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Although obesity is often associated with high-fat diets, it can develop from a variety of meal patterns. Excessive intake of simple carbohydrates is one consistent eating behavior leading to obesity. However, the impact of overconsumption of diets with high carbohydrate to fat ratios (C/F) on body composition and global adipose tissue gene expression remains unclear. We used total enteral nutrition to evaluate the effects of caloric intake and C/F on body weight gain and development of obesity. Female Sprague Dawley rats were fed diets with either low C/F or high C/F (HC) (reflecting a 19.5-fold increase in C/F) at two levels of caloric intake: 187 or 220 kcal/kg\(^{3/4}\)d (15% excess) for 4 wk. At the end of the study period, rats fed HC diets had about 20% higher body weight at either caloric intake compared with rats fed low C/F diets (\(P < 0.05\)). Body composition (assessed by nuclear magnetic resonance, computerized tomography, and adipose tissue weights) revealed higher percent fat mass (\(P < 0.05\)) in HC rats. Obesity was associated with increased serum resistin, leptin, fasting hyperinsulinemia, and insulin resistance after an oral glucose challenge (\(P < 0.05\)). Microarray analyses of adipose tissues revealed HC diets led to changes in 270 and 464 transcripts at 187 and 220 kcal/kg\(^{3/4}\)\cdot d intakes. Genes regulating glucose transport, glycolysis, fatty acid and triglyceride biosynthesis, desaturation and elongation, adipogenesis, and adipokines were affected by HC diets. These results suggest that C/F and interactions with excessive caloric intake per se may regulate body composition and play important roles in the development of obesity and metabolic syndrome. (Endocrinology 151: 153–164, 2010)

Dietary carbohydrates exert profound influence on several aspects of body weight accretion, endocrinology, and appetite (1, 2). Mechanisms to use carbohydrates directly for energy via oxidation (glycolysis) or energy storage via triglyceride synthesis (lipogenesis) are highly conserved. In mammals, both the liver and adipose tissues are equipped to carry out these processes (3, 4). After a high-carbohydrate meal, plasma glucose rises, triggering a rapid release of insulin from pancreatic \(\beta\)-cells. Insulin, mainly via the insulin receptor/phosphatidylinositol 3-kinase pathway, acts on its target tissues and orchestrates a multitude of anabolic effects: inhibiting gluconeogenesis in the liver, enhancing glucose uptake and oxidation in skeletal muscle, and suppressing lipolysis and increasing lipogenesis in adipose tissues (5). The lipogenic actions of insulin are in large part mediated via transcriptional activation of target genes controlled by sterol regulatory element binding protein (SREBP)-1c. In recent years, it has become evident that glucose itself is an important regulator of mRNA transcription of an increasing number of targets genes via the carbohydrate-response element binding protein (ChREBP) (6–11).

Abbreviations: ACC, Acetyl CoA carboxylase; ChREBP, carbohydrate response element binding protein; C/F, carbohydrate to fat ratio; CT, computerized tomography; GO, gene ontology; HC, high carbohydrate to fat ratio; H&E, hematoxylin and eosin; IPA, ingenuity pathway analysis; LC, low carbohydrate to fat ratio; NEFA, nonesterified fatty acid; NMR, nuclear magnetic resonance; OGTT, oral glucose tolerance test; PPAR, peroxisome proliferator-activated receptor; SREBP, sterol regulatory element binding protein; TEN, total enteral nutrition.
The majority of studies examining carbohydrate-induced transcriptional regulation have focused on the liver (7, 8, 11). Relatively little is known about the effect of diets with high carbohydrate to fat ratios (C/F) on global gene expression profiles in the adipose tissue (12). Furthermore, few studies have examined the effect of high C/F (HC) on development of obesity, insulin resistance, and other metabolic/endocrine parameters (13). Self-limiting consumption of diets due to satiety has been the primary limitation in examining the effects of overfeeding HC diets on development of obesity in animal models. In the present study, we used controlled enteral feeding of liquid diets via total enteral nutrition (TEN) as a mechanistic tool to overcome this limitation.

The present studies had two objectives. First, we examined the interaction between caloric intake and carbohydrate content on body weight and adiposity. Specifically, we examined how increasing dietary C/F, either in the context of normal or overfed states, impacts body weight and composition. Second, we elucidate global transcriptomic changes in the adipose tissues and the biochemical and endocrinological parameters, we assessed concentrations of adipose-related biochemical alterations that occur in response to high dietary carbohydrates. In addition, we examine the relationship between changes in body composition caused by HC diets and development of whole-body insulin resistance. Our data strongly suggest that HC diets transcriptionally regulate a diverse suite of genes that leads to coordinated regulation of glucose transport, glycolysis, and lipid biosynthesis in the adipose tissue leading to greater obesity and insulin resistance.

Materials and Methods

Animals and chemicals

Female Sprague Dawley rats (150–175 g) were purchased from Charles River Laboratories (Wilmington, MA). Animals were housed in an Association Assessment and Accreditation of Laboratory Animal Care-approved animal facility. Animal maintenance and experimental treatments were conducted in accordance with the ethical guidelines for animal research established and approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences. Unless otherwise specified, all chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

Experimental protocol

Rats were intragastrically cannulated and allowed to recover for 10 d as previously described (14–17). Rats (n = 7–9/group) were fed liquid diets via TEN. Diets were either low C/F [LC; 35% carbohydrate and 45% fat (corn oil), as percent of total calories] or HC (75% carbohydrate and 5% fat, as percent of total calories), reflecting a 19.5-fold increase in C/F in the HC group. Both diets were isocaloric, met National Research Council nutrient recommendations, including essential fatty acids, and provided 20% of total calories from protein (casein). Rats were fed via TEN for 23 h/d at either 187 kcal/kg1/3 · d (Research Council recommendations) or 220 kcal/kg1/3 · d (15% overfed). Compositions of diets have been described previously (14, 15, 17). Animals had unlimited access to drinking water throughout the experiment. TEN feeding continued for 4 wk throughout which body weights were monitored twice weekly. At the end of 4 wk of diet infusion, body composition was noninvasively estimated using nuclear magnetic resonance (NMR; Echo Medical Systems, Houston, TX) and x-ray computed tomography (CT; LaTheta LCT-100; Echo Medical Systems) as detailed below. At the end of the study, rats were euthanized under anesthesia and blood, livers, kidneys, and adipose tissues (periretioeleale, gonadal, and perirenal depots) were weighed and collected. Samples were fixed in alcohol-formalin for histological analyses, and remaining tissues were frozen in liquid nitrogen and stored at −70 C for RNA and protein analyses. Serum was obtained by centrifugation of blood samples and stored at −20 C for endocrine and metabolic assessments. In a separate set of animals (n = 5/group), which included an ad libitum chow-fed control group, insulin sensitivity was examined after an oral glucose tolerance test (OGTT).

Body composition analyses

Body composition was assessed via three independent methods, namely whole animal body composition by NMR (Echo Medical Systems), x-ray CT (LaTheta LCT-100; Echo Medical Systems) and postmortem dissected weights of retroperitoneal, perirenal, and gonadal fat pads from rats (17). NMR was performed in conscious rats and did not require the animals to remain still. Each NMR measurement took about 1 min/rat and all measurements were performed in duplicate (17). Indices of percent fat and lean mass were derived using this technique. For CT analyses, approximately 90 sections, 1 mm apart, were acquired encompassing the entire visceral region of the animal under isoflurane anesthesia. Densitometric calculations of fat and muscle were performed using Aloka CT software (Tokyo, Japan) using attenuation number thresholds of −120 to −500 for fat and −120 to +350 for muscle. Indices of percent fat mass and percent lean mass were calculated. Subcutaneous and visceral fat tissues were distinguished via manual tracing of the abdominal wall in each of the sections (17).

OGTT and other endocrine parameters

To assess development of insulin resistance in relationship with adiposity changes, rats were challenged with an OGTT. All rats were fasted for 6 h (from 0900 to 1500 h) before receiving a 3.5 g/kg challenge of glucose (0.5 g/ml solution) (17). Blood (~50 μl) from the tail vein was collected in capillary tubes at the beginning of the fast and at 0, 15, 30, 60, 90, and 150 min after the glucose challenge. Serum glucose was measured using glucose oxidase method (Synermed, Westfield, IN). Serum insulin concentrations were assayed using ELISA for rat insulin (Millipore, Billerica, MA). To determine status of adipose-related biochemical and endocrinological parameters, we assessed concentrations of serum leptin, adiponectin, resistin, nonesterified fatty acids (NEFAs), and triglycerides. Hormones were assayed using ELISA (leptin; Linco Research, St. Charles, MO; adiponectin and resistin; B-Bridge International, Sunnyvale, CA). Serum triglycerides were estimated using colorimetric assay (Synermed). NEFA levels were measured using NEFA C kit (Wako Chemicals, Richmond, VA).
Adipose gene expression analyses

RNA isolation and microarray analyses

Total RNA was isolated from retroperitoneal adipose tissues of rats (n = 7–9/group) fed LC or HC diets at 187 and 220 kcal/kg^{1/4}·d using TRI reagent (Molecular Research Center, Cincinnati, OH) and cleaned using RNeasy kit (QIAGEN, Valencia, CA). Three microarrays (GeneChip Rat 230 2.0) were used for each group. Pools of equal amounts of RNA from two to three rats per microarray were used for analyses. Thus, seven to nine rats per group were represented over the three microarrays. Briefly, 5 μg of purified RNA were used to synthesize cDNA. Labeled cRNA was synthesized from double-stranded cDNA using the GeneChip IVT labeling kit (Affymetrix, Santa Clara, CA) according to the manufacturer’s instructions. The probe array was scanned after the wash and staining protocols with GeneChip Scanner 3000 (Affymetrix). CEL files containing the 31,099 transcripts on the GeneChip Rat genome 230 2.0 array were generated using GCOS (Affymetrix).

Microarray data normalization and analysis

Microarray data analyses were carried out using GeneSpring version 7.3X software (Agilent Technologies, Santa Clara, CA) (18–20). The CEL files containing the probe level intensities were processed using the robust multiarray analysis algorithm (Agilent Technologies). The normalized data were subjected to pairwise comparisons as follows: 187-HC vs. 187-LC and 220-HC vs. 220-LC. In each comparison, genes were filtered based on minimum 1.8-fold change (HC vs. LC) and P ≤ 0.05 using Student’s t test. Corrections for multiple testing were performed using the False Discovery Rate (FDR) method (22). Volcano plots and a list of all transcripts that were differentially expressed as a function of HC was generated and correlation-based hierarchical clustering between treatment groups was performed. Known biological functions of genes were queried using Affymetrix NetAffx and gene ontology (GO) analyses performed using GeneSpring (Agilent Technologies) (18–20). Abbreviations for gene symbols can be queried from the NetAffx Data Analyses Center (http://www.affymetrix.com/analysis/index.affx). Furthermore, the list of genes affected by HC was analyzed using ingenuity pathway analysis (IPA).

Real-time RT-PCR

One microgram of total RNA was reverse transcribed (n = 7–9/group) using iScript cDNA synthesis kit, and subsequent real-time PCR analysis was performed using an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA). Gene-specific primers were designed using Primer Express Software (Applied Biosystems) (supplemental Table S1, published as supplemental data on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org). The relative amounts of mRNA were quantitated using a standard curve and normalized to the expression of cyclophilin A mRNA (18).

Histology and histomorphometry

Samples of liver tissues were fixed in either buffered formalin or optimum cutting temperature compound and processed using routine histological techniques (17, 20). For histomorphometric analyses of adipose tissue, 3- to 4-mm pieces from the retroperitoneal fat depots were fixed in buffered alcoholic formalin for 4 d and embedded in paraffin. Six-micrometer-thick sections were stained with hematoxylin and eosin (H&E). Diameters of adipocytes were measured using an Axiovert microscope (Carl Zeiss Inc., Thornwood, NY) with ZieissVision software (Carl Zieiss). A minimum of 300 cells at random were measured for each slide (n = 7–9/group) and percentage of cells in each size range was computed using MS Excel (Microsoft, Redmond, WA) (17, 20).

Cell lysate and immunoblotting

Total lysates from liver and retroperitoneal adipose tissues were prepared in radioimmunoprecipitation assay buffer containing 1 mM phenylmethylsulfonyl fluoride and protease inhibitor cocktail. Nuclear protein from adipose tissues was prepared using NE-PER reagents (Thermo Fisher, Rockford, IL). Proteins were resolved by SDS-PAGE and immunoblotting was carried out using standard procedures (17, 18, 23). Membranes were incubated with rabbit anti-FAS (Abcam, Cambridge, MA), rabbit anti-acetyl CoA carboxylase (ACC; Cell Signaling, Beverly, MA), rabbit anti-ChREBP (Cayman Chemicals, Ann Arbor, MI), antikine receptor β-actin, or lamin A (Sigma) antibodies for 16 h at 4°C. Proteins were visualized using ECL-Plus (GE Healthcare Bio-sciences, Piscataway, NJ) and detected by autoradiography followed by densitometric scanning.

Data and statistical analysis

Data are expressed as means ± SEM. Associations between the variables, serum leptin, insulin at 15 min after glucose challenge, and percent fat mass, respectively, were examined by linear regression. Similarly, linear regression was carried out between percent fat mass and normalized mRNA expression in adipose tissues for selected genes quantitated via real-time RT-PCR. The data were tested for equality of variance. A two-way ANOVA followed by all-pair wise comparison by the Student-Neuman-Keuls method was used to compare the effects of carbohydrate diets and caloric intake (overfeeding). P ≤ 0.05 was considered statistically significant. Statistical analyses were performed using SigmaStat 3.3 software (Systat Software Inc., San Jose, CA). Graphical representation performed using SigmaPlot version 10.0 for Windows (Systat Software).

Results

HC diets lead to changes in body weight, fat accretion, and endocrine profiles

Rats fed 220-kcal diets for 4 wk were heavier and exhibited greater adiposity compared with 187 kcal-fed counterparts (Fig. 1, A and B, P < 0.05). However, both at 187 and 220 kcal intakes, HC diets led to greater weight gain (125 and 116%) compared with rats fed LC diets (Fig. 1A, P < 0.05). Percent fat mass in HC-fed animals was 153 and 161% greater when compared with the LC diet-fed rats (Fig. 1B). Quantitation of percent fat mass and lean mass in the truncal region using CT revealed increased adiposity (P < 0.05) in both visceral and sc compartments.
(Fig. 1, C and D). Finally, postmortem quantitation of dissected fat depots showed a robust effect \((P < 0.001)\) of HC diets and overfeeding on total fat weights (retroperitoneal, gonadal, and perirenal depots) (Table 1). HC diets also significantly increased relative liver weights \((P < 0.001)\) (Table 1). In separate preliminary studies, we determined that body weight gain and body composition of rats fed 187-LC diets via TEN were similar to that of ad libitum chow-fed rats (body weight at 4 wk, 267 ± 16 in chow fed vs. 271 ± 4 in 187-LC, respectively; percent lean mass at 4 wk, 63 ± 0.7 in chow fed vs. 62.7 ± 1.1 in 187-LC, respectively; percent fat mass at 4 wk, 14 ± 1 in chow fed vs. 17 ± 1.1 in 187-LC, respectively). Both fed and fasting concentrations of insulin, leptin, and glucose were also not significantly different between the two groups (data not shown). Hence, the 187-LC fed rats reasonably serve as a lean control group to examine effects of HC diets.

The impact of overfeeding and HC diets on endocrine and biochemical parameters was investigated (Table 1). Serum glucose was only modestly increased by HC diets but robustly increased by overfeeding \(\textit{per se}\) \((P < 0.005)\). Serum insulin levels increased 300 and 130% at 187 and 220 kcal, respectively, in rats after HC diets, indicating development of insulin resistance. As anticipated, serum leptin levels positively correlated with degree of adiposity (Table 1, \(r^2 = 0.84, P < 0.0001)\). Moreover, despite normalization of serum leptin levels to fat mass, higher serum leptin concentrations were observed in the rats fed HC diets at 187 kcal, indicative of leptin resistance \((0.45 ± 0.03 vs. 0.74 ± 0.08 in \text{LC and HC-187 groups, respectively, } P < 0.01)\). Serum adiponectin concentrations were decreased by about 50% after overfeeding of LC diets. However, feeding of HC diets led to increased serum adiponectin levels at both caloric intakes \((P < 0.01)\), suggesting that carbohydrate-driven adiposity in this model was
specifically increased by overfeeding (P < 0.05; Fig. 2, A and B). At 187 kcal, the percentage of adipocytes in the 75- to 100-μm size range increased from 12% in LC rats to 27% in the HC-fed rats (P < 0.05). Similarly, feeding the HC diet at 220 kcal led to dramatic hypertrophy of adipocytes. At 220 kcal, approximately 33% cells were in the greater than 100 μm size range (P < 0.05) compared with 8% cells in the LC rats (Fig. 2B).

**HC diets lead to systemic insulin resistance**

To assess whether increased obesity and adipose hypertrophy in HC-fed rats were associated with insulin resistance, we performed OGTT. Rats fed the HC diets at 187 and 220 kcal showed fasting hyperinsulinemia (P < 0.05; Fig. 2C). Marked changes in serum insulin response were observed after glucose challenge along with the gain in body weight and development of adiposity. Overfed rats (both LC and HC) were more insulin resistant compared with controls. Whereas insulin responses of 187-LC rats were similar to chow-fed controls (supplemental Fig. S1), serum insulin levels were greater in HC-187 rats compared with LC controls (Fig. 2, D and E). Hyperglycemia was observed in the overfed HC group, suggesting worsening of the glucose intolerance phenotype (Fig. 2F). Correlation analyses of the peak insulin response (15 min) and percent fat mass (from NMR) revealed a strong positive relation analyses of the peak insulin response (15 min) and percent fat mass (from NMR) revealed a strong positive correlation (P < 0.0001, r² = 0.87, Fig. 2G), indicating that development of obesity was associated with progressive insulin resistance.

We further examined the status of two critical lipogenic enzymes in the liver and adipose tissues. Immunoblot analyses of total lysates revealed significant induction of both hepatic and adipose tissue, fatty acid synthase, and ACC-1 in HC-fed groups (Fig. 2, H and I). Furthermore, levels of ChREBP were significantly increased in adipose tissue nuclear extracts from HC-fed rats (Fig. 2J). These data are consistent with increased hepatic steatosis and adipose hypertrophy in the HC-fed rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>187 kcal/kg⁴/₃ · d</th>
<th>220 kcal/kg⁴/₃ · d</th>
<th>Effect of HC</th>
<th>Effect of overfeeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight at 4 wk</td>
<td>226 ± 6.1</td>
<td>266 ± 6.8</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percent liver weight</td>
<td>3.2 ± 0.06</td>
<td>3.5 ± 0.08</td>
<td>&lt;0.001</td>
<td>0.08</td>
</tr>
<tr>
<td>Percent total fat</td>
<td>1.5 ± 0.25</td>
<td>3.8 ± 0.15</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percent RP fat</td>
<td>0.58 ± 0.10</td>
<td>1.4 ± 0.08</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Percent gonadal fat</td>
<td>0.64 ± 0.13</td>
<td>1.7 ± 0.10</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percent perirenal fat</td>
<td>0.32 ± 0.06</td>
<td>0.68 ± 0.09</td>
<td>&lt;0.001</td>
<td>0.049</td>
</tr>
<tr>
<td>Percent kidney weight</td>
<td>0.72 ± 0.02</td>
<td>0.70 ± 0.02</td>
<td>0.16</td>
<td>0.76</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>110 ± 4.9</td>
<td>123 ± 8.4</td>
<td>0.06</td>
<td>0.005</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>1.7 ± 0.15</td>
<td>5.1 ± 1.0</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>8.0 ± 0.6</td>
<td>20.6 ± 2.6</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adiponectin (μg/ml)</td>
<td>18.1 ± 5.5</td>
<td>21.6 ± 8.0</td>
<td>0.01</td>
<td>0.57</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>39.9 ± 5.9</td>
<td>73.1 ± 18.4</td>
<td>0.02</td>
<td>0.57</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>63 ± 15</td>
<td>151 ± 13</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>NEFA (μm)</td>
<td>156 ± 12</td>
<td>233 ± 21</td>
<td>0.66</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Data were obtained at 4 wk after consumption of LC or HC diets at 187 or 220 kcal/kg⁴/₃ (n = 7, 9, 9, and 9 in LC and HC groups at 187 and 220 kcal, respectively). Rats were fed diets via TEN to as described in Materials and Methods. Data are expressed as means ± SEM. Statistical differences were determined using a two-way ANOVA to examine the effects of HC and overfeeding, followed by Student-Neuman-Keuls post hoc analyses. RP, Retroperitoneal adipose tissue.

not associated with hypoadiponectinemia. Serum resistin concentrations increased with HC diets at both caloric intakes (P < 0.05). Serum triglyceride levels were increased by HC (P < 0.05), and NEFA concentrations were specifically increased by overfeeding (P < 0.05) (Table 1).

**Histology and adipose histomorphometry**

Histological examination of H&E- and Oil Red O-stained liver sections revealed significant hepatic lipid accumulation in HC-fed rats (Fig. 2A). Overfeeding of LC diets caused a modest increase in hepatic lipid levels. Feeding of HC diets increased the mean diameter of retroperitoneal adipocytes, indicating hypertrophy (P < 0.05, Fig. 2, A and B). At 187 kcal, the percentage of adipocytes in the 75- to 100-μm size range increased from 12% in LC rats to 27% in the HC-fed rats (P < 0.05). Similarly, feeding the HC diet at 220 kcal led to dramatic hypertrophy of adipocytes. At 220 kcal, approximately 33% cells were in the greater than 100 μm size range (P < 0.05) compared with 8% cells in the LC rats (Fig. 2B).

**HC diets lead to coordinated transcriptomic changes in the adipose tissue**

Microarray data were analyzed after robust multiarray analysis normalization using GeneSpring Gx version 7.3 (Agilent Technologies). Unsupervised global condition clustering of microarrays revealed significant association of gene expression profiles between samples from the same group, indicating significant treatment effects before any group comparisons (supplemental Fig. S2A). Pairwise comparisons of 187-HC vs. 187-LC and 220-HC vs. 220-LC showed that 270 and 464 transcripts were altered, respectively (±1.8-fold, P ≤ 0.05, Fig. 3A). The resulting union of these comparisons containing 581 transcripts re-
flects the effect of HC diets at either caloric intake (supplemental Table S2). Two-way correlation-based hierarchical clustering of the carbohydrate-responsive genes is depicted in supplemental Fig. S2B. These transcripts were used for GO analyses based on molecular function, biological function-based hierarchical clustering, and pathway analyses. Most prominently altered genes were classified as having binding or catalytic functions representing 39 and 27% of the 581 transcripts altered by HC (Fig. 3B). Approximately 8% of genes possessed transporter or signal transduction functions and about 2.5% genes had bona fide roles in regulating transcription (transcription factors, coactivator/repressors) (Fig. 3B). Furthermore, we interrogated known biological functions of genes altered by HC. Of the 581 transcripts altered, 100 transcripts were expressed sequence tag sequences with poorly defined annotations. Approximately 55 transcripts were identified via the Rat Genome Database based on sequence similarity. Using GeneSpring Gx version 7.3 (Agilent Technologies), we identified 29 specific genes involved in carbohydrate metabolism and 16 genes with known roles in lipid biosynthesis (Fig. 3C and supplemental Table S2). Correlation-based hierarchical clustering of genes involved in carbohydrate metabolism and lipid biosynthesis is shown in Fig. 3, C and D. Additionally, we found suites of genes involved in regulating electron transport, adipocyte secretory products, and intracellular transport (supplemental Table S2). We further used GeneSpring Gx 7.3 (Agilent Technologies) and pathway analysis software (IPA) to identify common regulators of the altered genes. SREBP-1, ChREBP, and leptin were identified as critical nodes of regulation by HC diets, consistent

FIG. 2. A, Representative photomicrographs of liver and adipose tissues from rats fed diets with LC or HC via TEN at 187 kcal/kg0.75 (n = 7 and 9 in LC and HC, respectively) or 220 kcal/kg0.75 (n = 9 and 9 in LC and HC, respectively). a–d, H&E-stained liver sections; e–h, Oil-Red-O-stained adipose tissues. B, Estimation of adipocyte size in the retroperitoneal adipose tissue of rats fed 187 kcal/kg0.75 (top panel) or 220 kcal/kg0.75 (bottom panel) (n = 7–9/group). Diameter of a minimum of 300 adipocytes at random was estimated, magnification, ×100. Data are expressed as the average (±SEM) percentage of adipocytes in a given size range. Differing superscripts indicate significant differences (P < 0.05). Serum insulin (C and D) and glucose (E and F) after an oral glucose challenge (2.5 g/kg) to fasted rats fed LC or HC diets via TEN for 4 wk. Statistical differences were determined using a Student’s t test at each time point. *, P < 0.05 compared with lean rats at the same time point. G, Linear regression analyses between percent fat mass derived via NMR analyses and serum insulin at 15 min after oral glucose challenge in rats fed LC or HC diets via TEN for 4 wk. Immunoblot analyses of fatty acid synthase and ACC protein expression in hepatic (H) and adipose (I) tissue total lysates from rats fed LC or HC diets via TEN for 4 wk. J, Immunoblot analyses of ChREBP and lamin A protein expression adipose tissue nuclear extracts from rats fed LC or HC diets for 4 wk.
FIG. 3. A, Venn diagram of differentially expressed adipose tissue genes at 187 kcal/kg^{3/4}·d or 220 kcal/kg^{3/4}·d after HC diet. Genes were filtered based on minimum ±1.8-fold change (HC vs. LC) and P value ≤0.05 using Student’s t test. B, Pie chart of GO molecular function of differentially expressed transcripts by HC diets at either caloric intake. C and D, Two-way hierarchical clustering of genes with specific functions in carbohydrate or lipid metabolism derived from the list of HC altered genes. The heat map was generated using GeneSpring Gx version7.3 (Agilent Technologies). Colors orange, yellow, and blue represent up-regulation, no relative effect, and down-regulation of adipose tissue genes, respectively. E, IPA gene network of highest significance identified using IPA software from the list of HC altered genes. Interactions between SREBP-1, ChREBP (MLXIPL), Glut4 (SLC2A4), and leptin signaling with several lipogenic targets is evident. Colors green and red represent down-regulation and up-regulation, respectively.
with increased adipose hypertrophy and expression of lipogenic genes (Fig. 3E).

We performed independent verification of 25 genes using real-time RT-PCR (Fig. 4). To further understand the physiological changes in the adipose after HC diets, we included important candidates whose mRNA expression was not changed in the microarray analyses (at the set thresholds of ±1.8-fold) (Fig. 4). These included adipogenic genes, inflammatory cytokines, and other genes involved in fatty acid metabolism (Fig. 4). Linear regression of mRNA expression for selected genes with percent fat mass (derived from whole body NMR) was carried out (supplemental Fig. S4). A summary of significant HC-induced gene expression changes of selected biological functions is described below.

**Carbohydrate metabolism**

Expression of genes involved in carbohydrate transport (Glut4), glycolysis (hexokinase, Aldo2), malonyl CoA, and precursor biosynthesis (Me1, ATP-citrate lyase, OCD, acetoacetyl CoA synthase, acetyl CoA carboxylase; supplemental Table S2) and mitochondrial decarboxylate import were significantly induced by carbohydrates. In addition, expression of genes regulating the pentose phosphate pathway (G6PD, transketolase) was transcriptionally increased in the adipose tissue after HC. Previous studies implicated activation of ChREBP, via increased glucose flux through the pentose phosphate pathway through xylulose-5-phosphate (8). Our results show that HC diets also transcriptionally activate ChREBP mRNA in adipose tissues (Fig. 4 and supplemental Table S2), consistent with recent studies in primary hepatocytes (11).

**Fatty acid, triglyceride, and cholesterol biosynthesis**

Consistent with increased glycolytic flux into fatty acid biosynthesis, several genes regulating fatty acid biosynthesis, elongation, desaturation, and transport (FASN, Theddc1, Adiponutrin, Scd2, Elovl4, Elovl5 and Elovl6, Fads2, FABP1 and -5) were induced in rats fed HC diets. Adiponutrin, which has been shown to be regulated by glucose in liver and adipose tissues, was induced about 3- to 5-fold at both adequate and overfed caloric intakes (11, 24). Several genes involved in triglyceride biosynthesis (G3PD, AGPAT2, AGPAT3, DGAT1) were also increased after HC diets. In addition, we observed a coordinated induction of 13 genes involved cholesterol biosynthesis

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**FIG. 4.** A and B, mRNA expression of genes in retroperitoneal adipose tissues of rats fed LC or HC via TEN at 187 kcal/kg^{3/4}·d (n = 7 and 9 in LC and HC, respectively) or 220 kcal/kg^{3/4}·d (n = 9 and 9 in LC and HC, respectively). Gene expression was assessed via real-time RT-PCR. Expression of each gene was quantitated on a standard curve and normalized to the expression of cyclophilin A. Data are expressed as means ± SEM. Statistical differences were examined using a two-way ANOVA for the effects of HC and overfeeding, followed by Student-Neuman-Keuls post hoc analyses. Overall P values for each effect are given below the respective histogram.
and transport, including key genes in this pathway (Mvd, Lss, Sc5d, CYP51, DHCR7, ApoAI and -AII) (supplementary Fig. S3).

**Transcription factors**

HC diets potently induced mRNA expression of several transcription factors including CRE modulator (CREM), Forkhead A1, ChREBP, SREBP-1, and the basic helix-loophelix B2 (Bhlhb2) proteins (Fig. 4 and supplemental Table S2). Real-time RT-PCR revealed that the expression of adipogenic transcription factors peroxisome proliferator-activated receptor (PPAR)-γ2 and CCAAT/enhancer-binding protein-α was significantly induced by HC diets (P < 0.05), suggesting that compensatory adipogenesis was induced after HC diets. However, gene expression of PPAR-α and -δ was not altered by HC diets (Fig. 4).

**Inflammatory cytokines and other adipocyte-secreted factors**

Consistent with increased fat mass and serum leptin levels, mRNA of leptin was increased with both HC diets and overfeeding (P < 0.01, Fig. 4). However, we also identified several novel factors that were altered by HC diet and overfeeding. These included four serine proteinase inhibitors (serpin) isoforms (A1, A7, A12, C1) that were induced by HC and Serpin12 that was robustly down-regulated by HC diets. In addition, we observed down-regulation of Wnt genes (Wnt2a and -4) in the adipose tissue (supplemental Table S2). Finally, we examined expression of inflammatory cytokines using real-time RT-PCR. Expression of TNF-α, IL-6 or monocyte chemotactic protein-1 was not altered by either HC diet or overfeeding (Fig. 4).

**Discussion**

Both the total caloric intake and percentage of calories derived from carbohydrates in the American diet have steadily increased over the last 30 yr (25, 26). Studies in human subjects as well as animal studies (in vivo and in vitro) clearly indicate that overconsumption of HC diets modulate cellular pathways controlling de novo lipogenesis (13, 27–31). In an isocaloric diet, studying macronutrient caloric sources necessitates alterations in relative ratios of macronutrients. The present study is the first to comprehensively address adipose tissue gene expression in rats fed different dietary C/F and maintaining constant protein calories. For ease of discussion, we use HC and LC as abbreviations for high and low C/F, respectively. Several novel findings are evident from these studies: 1) HC diets led to greater adiposity than LC diets at two levels of caloric intake, 2) overconsumption of calories irrespective of diet composition results in body fat accretion, hyperinsulinemia, and insulin resistance, albeit leading to markedly varied adipose gene expression signatures, and 3) despite the low total amount of dietary fat in the HC diets, adiposity after overfeeding of HC diets was associated with metabolic derangements akin to models of either genetic or high-fat diet-induced obesity.

HC diets induce expression of genes involved in glucose uptake, glycolysis, and lipogenesis in a ChREBP-dependent fashion (8, 11). Upon activation by high glucose levels, the ChREBP, Mlx heterodimer mediates transcription of target genes through carbohydrate response element elements. Mice lacking ChREBP are intolerant to simple carbohydrates, present decreased expression of the glycolytic enzyme 1-pyruvate kinase, and have excess hepatic glycogen due to inability to metabolize glucose (32). Most importantly, ChREBP is required for the normal lipogenic response after a carbohydrate load (32). Whereas ChREBP is highly expressed in the liver, it is also expressed in adipose tissues, intestine, and pancreatic islets. Expression of ChREBP is induced in 3T3-L1 cells during differentiation, and ChREBP knockout mice have smaller adipose tissue mass on a high carbohydrate diet, suggesting that ChREBP may also control lipogenesis in the adipocyte (32, 33). Similar to the response in the liver, insulin-responsive induction of lipogenic genes in adipocytes is greatly enhanced by higher ambient concentrations of glucose (12). However, it remains unknown whether ChREBP mediates induction of lipogenic genes in the adipocyte. Our results from the present study establish that a HC diet positively regulates adipose tissue ChREBP mRNA and protein in vivo and other enzymes involved in the pentose phosphate pathway (transketolase, glucose-6-phosphate dehydrogenase). These enzymes collectively provide the reduced form of nicotinamide adenine dinucleotide phosphate (NADP+), required for fatty acid biosynthesis and generate xylulose-5-phosphate, leading to activation of ChREBP.

Recently elegant studies by Ma et al. (11) used microarrays and identified the profile of glucose-responsive genes in primary hepatocytes. These studies confirmed that ChREBP, Mlx is critical in mediating glucose-regulated gene expression. Results from our gene expression analyses are remarkably similar to findings from these experiments. Expression of GLUT4, Aldolase B, G6PD, transketolase, Me1, glycogen synthase 2, ATP-citrate lyase, FASN, ACC-1, AACS, G3PD, Dhcr7, Adiponutrin, AdipoR2, ChREBP were some of the key genes commonly induced by HC diets in the adipose tissue, reported to be increased in a ChREBP-dependent manner. Our results suggest that the adipose response is similar to liver, a finding that is not altogether surprising, considering that in-
sulin and glucose enhance lipogenesis in both tissues. However, it appears that the carbohydrate-responsive gene expression profile in the adipose tissue is much greater than previously appreciated. Genes in the entire pathway of glycolysis, pentose phosphate pathway, fatty acid and triglyceride biosynthesis, and desaturation/elongation and lipolysis are affected by dietary carbohydrates in vivo (Fig. 5).

The present data also identified several novel carbohydrate-responsive targets in vivo unique to adipose tissue. Expression of several SREBP-1c target genes such as ELOVL6, SCD1 and -2, FABP5, and suite of genes controlling cholesterol biosynthesis and transport were elevated by overfeeding of HC diets. mRNA expression of several lipogenic genes correlated significantly with percent fat mass (supplemental Fig. S4). Expression of adipokines leptin, SerpinA12 (regulating insulin sensitivity), haptoglobin (acute phase response protein), and IGF binding protein-3 among others was also altered. Pathway analyses revealed that SREBP-1, ChREBP, and leptin were major regulators of the observed gene expression profile. These results differ from in vitro studies because overfeeding of LC or HC diets in vivo concomitantly increases serum insulin. Previous studies have shown that overfeeding of mice also causes an increase in SREBP-1 in the adipose tissue (34).

Changes in several metabolic and endocrine parameters were diet composition dependent. Serum triglycerides were elevated by overfeeding carbohydrates, whereas NEFA levels increased more robustly as a function of overall caloric excess. These findings are consistent with increased hepatic de novo lipogenesis, steatosis, and increased secretion of hepatic very low-density lipoprotein, resulting in higher serum triglyceride after HC feeding (35). Higher NEFA levels, on the other hand, presumably result from increased lipolysis of dietary fat in the LC diets. Adiponectin levels were decreased specifically by overconsumption of LC diets, consistent with several lines of evidence showing high-fat diets, and more specifically free fatty acids, may be the primary culprits in reducing adiponectin levels (36). Despite these differences, significant insulin resistance that correlated with increased fat mass developed with obesity.

Previous studies highlighted a role for adipose tissue inflammatory gene expression in mediating insulin resistance (36, 37). In addition, adipocytes activation of toll-like receptor and c-Jun N-terminal kinase signaling have been implicated in this process (38, 39). However, we
did not find increases in gene expression of inflammatory cytokines, TNF-α, IL-6, and monocyte chemotactic protein-1, presumably because of the relatively short duration of obesity (4 wk), compared with 8–24 wk in standard diet-induced obesity models. Hence, it appears that insulin resistance after HC-induced obesity develops via pathways independent of inflammation. Other literature suggests that hypertrophic adipocytes are per se less insulin sensitive (40, 41). Because HC diets lead to dramatic hypertrophy of adipocytes, mechanisms related to this may be important. Furthermore, because skeletal muscle insulin action is by far the most important determinant of whole-body insulin sensitivity, changes in endocrine profiles such as increases in serum resistin after HC diets may also play an important role. Additionally, we observed increases in expression of adipogenic genes, PPAR-γ2 and CCAAT/enhancer-binding protein-α, suggesting increased adipogenesis in HC-fed rats. Consistent with this, components of the Wnt pathway (Wnt2b, Wnt4) that normally antagonize adipogenesis were down-regulated in HC-fed rats (supplemental Table S2). Because TNF-α expression also antagonizes adipogenesis (42), it is likely that the lack of increases in TNF-α along with lower Wnt expression, allows for differentiation of preadipocytes to occur.

In conclusion, we have demonstrated that consumption of nutritionally complete diets with high C/F in a controlled manner results in extensive gene expression changes in the adipose tissues. Increased expression of a number of genes regulating glycolysis and lipid biosynthesis appears to be coordinated via the lipogenic transcription factors SREBP-1 and ChREBP. The increased lipogenic drive is reflected in and correlated with greater accretion adipose mass and the concomitant development of insulin resistance and metabolic dysfunction. These results suggest that targeting carbohydrate-regulated lipogenic pathways may be an effective strategy in mitigating increased adiposity in at least a subset of the population.

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